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Figure 16 illustrates the sequences of TGF-β superfamily members aligned using the Clustal method, from the first canonical framework cysteine to the end of the sequence for transforming growth factor-β1 (TGFβ1), transforming growth factor-β2 (TGFβ2), transforming growth factor-β3 (TGFβ3), inhibin β A (INHβA), inhibin β B (INHβB), the *nodal* gene (NODAL), bone morphogenetic proteins 2 and 4 (BMP2 and BMP4), the *Drosophila decapentaplegic* gene (dpp), bone morphogenetic proteins 5-8 (BMP5, BMP6, BMP7 and BMP8), the *Drosophila* 60A gene family (60A), bone morphogenetic protein 3 (BMP3), the Vg1 gene, growth differentiation factors 1 and 3 (GDF1 and GDF3), dorsalin (drsln), inhibin α (INHα), the *MIS* gene (MIS), growth factor 9 (GDF-9), glial-derived neurotropic growth factor (GDNF) and neurturin (NTN);

Figure 17 illustrates (A) full length murine persephin gene (SEQ ID NO:177) with arrows indicating an 88 nt intron from positions 155-242 and (B) the nucleotide sequence of murine pre-pro persephin (SEQ ID NO:179) with encoded amino acid sequence (SEQ ID NO:185);

Figure 18 illustrates (A) full length rat persephin gene (SEQ ID NO:188) with arrows indicating an 88 nt intron from positions 155-242 and (B) the nucleotide sequence of rat pre-pro persephin (SEQ ID NO:190) with encoded amino acid sequence (SEQ ID NO:196);

Figure 19 illustrates a western blot analysis using anti-persephin antibodies to detect persephin protein in cell lysates from COS monkey cells transfected with the murine persephin gene (lane 2) or the rat persephin gene (lane 3) compared to cells transfected. with the non-recombinant vector alone (pCB6, lane 4) and the mature protein produced by *E. Coli* (lane 1);

Figure 20 illustrates the murine chimeric molecules (A) PSP/NTN containing the persephin fragment (residues 1-63) and the neurturin fragment (residues 68 100)(SEQ ID NO:141) and (B) NTN/PSP containing the neurturin

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fragment (residues 1-67) and the persephin fragment (residues 64-96)(SEQ ID NO:146) with the arrow indicating the crossover point in each;

Figure 21 illustrates the survival promoting effect of persephin in murine embryonic day-14 mesencephalic cells cultured for three days (a) in the absence of persephin where almost all of the cells are dead and (b) in the presence of persephin (100 ng/ml) where substantial neuronal cell survival is evident;

Figure 22 illustrates the survival promoting effect of persephin (PSP) in murine embryonic day-14 mesencephalic cells compared to effects of neurturin (NTN) and GDNF, measured by the number of cells stained with tyrosine hydroxylase (TOH);

Figure 23 illustrates RT/PCT survey for persephin expression in adult mouse tissues showing persephin expression by Kidney cells; and

Figure 24 illustrates the cDNA sequence of human pre-pro persephin (SEQ ID NO:203) with two silent mutations indicated at positions 30 and 360 and the encoded amino acid sequence (SEQ ID NO:217) with the first amino acid of the pro- region indicated by the double asterisks (**) at amino acid position 24 and the first amino acid of mature human persephin indicated by the single asterisk (*) at amino acid position 61.

Description of the Preferred Embodiments

The present invention is based upon the identification, isolation and sequencing of a DNA molecule that encodes a new growth factor, persephin. Persephin promotes cell survival and, in particular, the survival of neuronal cells. Prior to this invention, persephin was unknown and had not been identified as a discrete biological substance nor had it been isolated in pure form.

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neurturin and persephin make possible this powerful new approach which can now successfully identify other gene family members. Using this new approach, one may screen for genes related to GDNF, neurturin and persephin in sequence homology by preparing DNA or RNA probes based upon the conserved regions in the GDNF and neurturin molecules. Therefore, one embodiment of the present invention comprises probes and primers that are unique to or derived from a nucleotide sequence encoding such conserved regions and a method for identifying further members of the neurturin-persephin-GDNF gene family.

Conserved-region amino acid sequences have been identified herein to include Val -Xaa_1-Xaa_2-Leu-Gly-Leu Gly-Tyr where Xaa_1 is Ser, Thr or Ala and Xaa_2 is Glu or Asp (SEQ ID NO:108); Glu-Xaa_1-Xaa_2-Xaa_3-Phe-Arg-Tyr-Cys-Xaa_4-Gly-Xaa_5-Cys in which Xaa_1 is Thr, Glu or lys, Xaa_2 is Val, Leu or Ile, Xaa_3 is Leu or Ile, Xaa_4 is Ala or Ser, and Xaa_5 is Ala or Ser, (SEQ ID NO:113); and Cys-Cys-Xaa_1 Pro-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Asp-Xaa_6-Xaa_7-Xaa_8-Phe-Leu-Asp-Xaa_9 in which Xaa_1 is Arg or Gln, Xaa_2 is Thr or Val or Ile, Xaa_3 is Ala or Ser, Xaa_4 is Tyr or Phe, Xaa_5 is Glu, Asp or Ala, Xaa_6 is Glu, Asp or no amino acid, Xaa_7 is val or leu, Xaa_8 is Ser or Thr, and Xaa_9 is Asp or Val (SEQ ID NO:114). Nucleotide sequences containing a coding sequence for the above conserved sequences or fragments of the above conserved sequences can be used as probes. Exemplary probe and primer sequences encoding amino acid sequences of SEQ ID NOS:125-129; primers whose reverse complementary sequences encode amino acid sequences SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:130; and, in particular, nucleotide sequences, SEQ ID NOS:115-124. Additional primers based upon GDNF and neurturin include nucleic acid sequences encoding amino acid sequences, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:40 and SEQ ID NO:41; primers whose reverse complementary sequences encode SEQ

expressing the persephin gene. Certain tissues such as those identified below in example 18 have been found to express the persephin gene. The method comprises hybridizing a polynucleotide probe to mRNA from a sample of tissues that normally express the persephin gene or from a cDNA produced from the mRNA of the sample. The sample is obtained from a patient suspected of having an abnormality in the persephin gene or from a particular patient tissue or cell type suspected of having an abnormality in the persephin gene. The reference persephin polynucleotide probe can comprise a cDNA encoding the complete mouse persephin open reading frame (SEQ ID NO:179, or the complement thereof, SEQ ID NO:180); a cDNA encoding the complete rat persephin open reading frame (SEQ ID NO:190 or the complement thereof, SEQ ID NO:191); cDNAs encoding complete human persephin open reading frames (SEQ ID NOS:203 or 205, or their respective complements, SEQ ID NOS:204 or 206) or derivatives thereof or fragments thereof so long as such derivatives or fragments specifically hybridize to persephin mRNA or from a cDNA produced from a persephin mRNA.

To detect the presence of mRNA encoding persephin protein, a sample is obtained from a patient. The sample can be from blood or from a tissue biopsy sample. The sample may be treated to extract the nucleic acids contained therein. The resulting nucleic acid from the sample is subjected to gel electrophoresis or other size separation techniques.

The mRNA of the sample is contacted with a nucleic acid serving as a probe to form hybrid duplexes. The use of a labeled probes as discussed above allows detection of the resulting duplex.

When using the cDNA encoding persephin protein or a derivative of the cDNA as a probe, high stringency

conditions can be used in order to prevent false positives, that is the hybridization and apparent detection of persephin nucleotide sequences when in fact an intact and functioning persephin gene is not present. When using sequences derived from the persephin cDNA, less stringent conditions could be used, however, this would be a less preferred approach because of the

FIGURES AS AMENDED

FIGURE 20A

PSP/NW (SEQ ID NO: 137)

ALAGSCRLWSLTLPVAELGLGYASEEKVIFRYCAGSCPQEARTQHSLVLA 50
↓
RLRGRGRAHGRPCCRPTAYEDEVSFLDVHSRYHTLQELSARECACV 96

FIGURE 20B

NTH/PSP (SEQ ID NO:146)

PGARPCGLRELEVRVSELGLGYTSDETVLFRYCAGACEAAIRIYDLGLRR 50
↓
LRQRRRVRRERARAHPCCQPTSYADVTFLDDQHHWQQLPQLSAAACGCGG 100